Mysteries of prion diseases: transmission and biosafety

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Prion Diseases [aka Transmissible Spongiform Encephalopathies (TSEs)]

- Scrapie
- Bovine Spongiform Encephalopathy
- Transmissible Mink Encephalopathy
- Creutzfeldt-Jakob Disease
- Variant Creutzfeldt-Jakob Disease
- Fatal Familial Insomnia
- Gerstmann-Straussler-Scheinker Syndrome
- Kuru
- Feline Spongiform Encephalopathy
- Chronic Wasting Disease
Prion Diseases: aka Transmissible Spongiform Encephalopathies (TSEs):

**Animals:**
- Scrapie of sheep
- Bovine spongiform encephalopathy (BSE)
- Feline spongiform encephalopathy (FSE)
- Transmissible Mink Encephalopathy (TME)
- Chronic wasting disease (CWD) of cervids

**Humans:**
- Creutzfeldt-Jakob Disease (CJD)
- Kuru
- Fatal Familial Insomnia (FFI)
- Gerstmann-Straussler-Scheinker Syndrome (GSSS)
- Variant Creutzfeldt-Jakob Disease (vCJD)
Kuru discovered in New Guinea by Carlton Gadjusek, MD
The connection of Kuru to scrapie as TSEs made by William Hadlow, DVM

Kuru

by C. Gadjusek

Scrapie

by G.A. Wells and S.A.C. Hawkins
Early prion transmission studies

Very long incubation periods, but amazingly, cross species transmission
Prion protein: normal protein and proteinaceous infectious particle

Figure 2 (a) Scheme of the primary structure of the cellular prion protein and its posttranslational modifications. A secretory signal peptide resides at the extreme N terminus. The numbers describe the positions of the respective amino acids. The proteinase K (PK)-resistant core of PrPSc is depicted in gray; the approximate cutting site of PK within PrPSc is indicated by arrows. CC (pink), charged cluster; HC (green), hydrophobic core; S-S, single disulfide bridge; MA, membrane anchor region; GPI, glycosyl phosphatidyl inositol; CHO, facultative glycosylation sites; NMR, nuclear magnetic resonance.
Prion conversion (more B movie vs. religious)

- PrP<sup>C</sup> (PrPC)
  - Soluble
  - Alpha helical
  - GPI-linked
  - Protease-sensitive
  - Highly conserved
  - 253-6 aa
  - Function still uncertain

- PrP<sup>RES</sup> (PrPRES)
  - Insoluble
  - High β-sheet content
  - Protease-resistant
  - Cofactor molecules in conversion still elusive

- PrP<sup>RES</sup> oligomer
- Spontaneous or enciphered

- PrP<sup>RES</sup> fibrils w beta sheet core
Discovery of prions: the subject of two Nobel prizes: C. Gajdusek and S. Prusiner
Transmission of prion diseases

• Kuru: ritualistic cannibalism

• sCJD: iatrogenic, tissue grafts, and unknown

• BSE, FSE, TME, vCJD: ingestion of prion-contaminated animal products

• CWD, scrapie: horizontal -- saliva, urine, feces, environment

• ....but origin of the initial prions assumed to reflect spontaneous misfolding events
TSE’s: spongiform brain lesions associated with PrP\textsuperscript{RES}

CWD

deer brain

Neuron with vacuole

\textbf{PrP}\textsuperscript{RES}

PrP\textsuperscript{RES} in nucleus of vagus nerve

[^1]: Image sources as per referenced literature.
Prion disease diagnosis
Prion detection

- Clinical signs, CT & MRI patterns
- Detection of protease resistant PrP ($\text{PrP}^{\text{RES}}$)
  - Western blot
  - Immunohistochemistry
  - ELISA
- Detection of infectious prions:
  - Bioassay in animals
    - Mice, or more recently, PrP transgenic mice
  - Evolving in vitro assays
    - Seeding (PrP conversion)
    - Cell culture infection
Detection of PK-resistant PrP^{RES} by western blotting

Deer brains

<- Protease digestion
<- CWD+?
Prion diseases and the major human neurodegenerative diseases are linked by protein misfolding as a central process:

- Alzheimer’s disease (AD) – Abeta, Tau
- Parkinson’s disease (PD) – Alpha synuclein
- Amyotrophic lateral sclerosis (ALS) – SOD1, TDP43
- Fronto-temporal dementia/head trauma -- TPD43, Tau

All are characterized by polymerized misfolded fibrillar protein deposits in the brain

The mechanism of protein mis-folding is likely similar to that in prion diseases
Prion biohazards: exposure

• Contact transmission – no, not for human prions (we think)

• Food, supplements – yes (BSE)

• Blood, tissue transplant. – yes (sCJD, vCJD)

• Surgical instruments – yes (vCJD)

• Environment – ?low for humans, but yes for animals due to persistence
Prion biohazards: blood transfusion

- In humans shown only for vCJD (BSE), not for sCJD. Preclinical donors.
- In animals, shown for deer and sheep. As little as 200µl of whole blood (scrapie)
- Prions are mostly WBC- and platelet-associated – leucodepletion used for all transfusions in UK
- Estimated in UK 1/2000 people may be carrying vCJD*

*UK Blood Transfusion & Tissue Transplantation Services
Prion infectivity and biosafety

• Not inactivated by conventional agents—formalin, alcohols, autoclaving at 122°C
• Adhere to and persist on most surfaces, esp. metals, clay, silica, plastic
• Can persist in environment -- years
• Aerosolization? Not intensively studied thus far
• Minimum mucosal and IV infectious dose is unclear
• Species barrier occasionally breached
Prion biosafety: inactivation

- 1N NaOH
- 20,000 ppm (2%) NaOCl
- Autoclaving at 134°C
- 1% SDS + 0.1% HAc or NaOH (≥0.3%), 5-15 min. (i.e. SDS + high or low pH)
- Adding liquid phase autoclaving at 121°C to the above
- Strong phenolic disinfectants (toxicity) or vaporized H₂O₂ reduce titer 4-5 logs
- Steel wires contaminated with 263K hamster scrapie is the most standard assay system
Like viruses, prions are usually species specific.

**PrP^C<->PrP^{RES}** sequence homology closely related to susceptibility

- Transgenic expression of heterologous species PrP^C confers susceptibility of that species to that prion.

Species crossing demonstrated experimentally.

Indications of species barrier strength:

- Incubation time (long vs. short) and attack rate (low vs. high)
- Oral/nasal (natural) route susceptibility

Still, species jumping occurs in nature—likely rarely and unpredictably.
Prion biosafety in lab: BSL2 (++)

BioBubble™ laminar flow containment of equipment and reagents for cross-species amplification or any BSE-related seeds. Closed transfer to biosafety cabinets
Chronic wasting disease: a prion disease of cervids

Occurrence: 22 States
Greatest Prevalence:
CO/WY deer: 5-10%
RMNP elk: 11-15%
WI: deer 7.5%
CWD Clinical disease

CWD negative mule deer

CWD positive mule deer

Behavioral changes, weight loss, ear, head position, gait change, stereotypic behaviors, polydipsia, polyphagia, staring, incoordination, digestive dysfunction, salivation

Courtesy of Dr. Michael Miller, CO Div. Parks & Wildlife
CWD detection:

**Medulla, obex**

- Intracellular vacuoles
- PrP\(^{CWD}\) plaques
- Spongiform encephalopathy

**CWD status**

| PK     | - | + | - | - |

Ante-mortem detection based on lymphoid tropism--tonsil or rectal tissue

**PrP\(^{CWD}\) in follicles**
1. CWD transmission and pathogenesis:
   - Why/how is CWD transmitted so efficiently?
   - How do CWD prions enter, traffic, and exit the body across mucous membranes?
   - Prion aerosol infection?
   - Repeated low dose exposure?
   - Do tissue-specific prion variants exist?

2. Species/transmission barrier:
   - Are sympatric predator and contact non-cervid species susceptible to CWD prions?
   - Does trans-species infection alter the species barrier?
   - Can this be predicted in vitro?
3. In vitro detection/conversion assays:

- Protease-sensitive infectious prions
- More sensitive detection/diagnosis?
- Prion cross seeding—human AD/PD/ALS?
- Screen drugs and inactivators
- Process of conversion/amplification

4. Prion vaccination:

- PrPc immunization with a *Salmonella* vector
- Point of exposure longitudinal studies, serial collections
- CWD-free, hand-raised, human- and indoor-adapted deer
- Whitetailed deer fawns sourced from Univ of Georgia

We work with CWD as if has potential to infect humans
CWD prion transmission and shedding: general study design

- CWD+ donors
- Blood
- Saliva
- Urine/feces
- Brain/other
- Neg controls

Tonsil and rectal tissue biopsies to detect infection

- Tissues, body fluids & excreta
  - QuIC
  - PMCA
  - Bioassay
How are CWD prions shed and transmitted in deer?

- Oral infection
- Mother-to-offspring transmission
- Direct or indirect horizontal transmission

Prion peripheralization and excretion:
- Brain
- Conjunctiva
- Nasal mucosa
- Oral/tongue
- Salivary glands
- Lymph nodes
- Blood
- Kidney
- Ureter
- Urinary bladder
- Bone marrow
- Rectum
- Intestine
RT-QuIC detection of CWD prions in deer blood

Thioflavine T fluorescence

ThT Fluorescence Output

Time in hours

rPrP substrate

(+)-Deer1
(-)-Deer1
(+)-Deer3
(-)-Deer3
(+)-Deer2
(-)-Deer2
(+)-Deer4
(-)-Deer4
(+)-Deer5
(-)-Deer5
(+)-Deer6
(-)-Deer6
RT-QuIC detection of prions in deer saliva

Saliva from Deer #
- 133
- 136
- 144
- 776
- 778
- 781
- 782
- 785
- 813
- 815

Thioflavine T fluorescence

Fluorimeter/shaker
rPrP substrate

Time (hours)
Are prions transmissible by aerosol?  Yes

Chamber for nose-only exposure of cervid PrP transgenic mice
Some findings with CWD (and scrapie) prions:

1. Infectious prions are in saliva, blood, urine, muscle, feces
   – Detected by bioassay and new in vitro methods
   – Most tissues contain prions

2. Environmental/fomite transmission without animal to animal contact proven

3. CWD prions are shed from mucosal excretory/secretory tissues. Perhaps adapted for efficient mucosal infection?

4. CWD are transmissible by aerosol and through minor oral lesions
1. CWD efficient transmission:
   - Effective prion peripheralization
   - Transfer to body fluids and excreta
   - Trans-mucosal shedding
   - Mucosal crossing to establish infection

2. CWD species barrier:
   - Sympatric and/or predator non-cervid species susceptible to CWD?
   - Alteration of the CWD host range?
   - Adaptation -> peripheralization -> shedding/horizontal transmission?
Loveland CO (leaving home in AM....)
Summary re. CWD prion species barrier:

1. Some sympatric non-cervid species are susceptible to CWD (by exp. inoculation)
   • Voles, *Peromyscus spp* mice, ferrets, hamsters, cats

2. Trans-species amplification of CWD prions (in vivo or in vitro) generates infectious prions with altered host range

3. Trans-species adaptation is associated with oral susceptibility and alteration of TSE phenotype
Summary re. prion biosafety:

1. Prions are misfolded cellular proteins that can also become infectious agents
2. Prions adhere to most surfaces and are difficult, but not impossible, to inactivate completely
3. Species crossing by prions is rare, but also remains unpredictable
4. Trans-species adaptation may be associated with alteration of species barrier
5. Prion-like protein misfolding is associated with the major human neurodegenerative diseases
People responsible for CWD work:

Nicholas Haley

Timothy Kurt

Davis Seelig

Christina Sigurdson

UCSD

Davin Henderson

Amy Nalls

Candace Mathiason

Mark Zabel

Glenn Telling
trip home up driveway...